

CLAIMS

1. Method for identifying markers of the locus of a major resistance gene to RYMV, comprising:
- selective amplification of rice DNA fragments firstly from resistant individuals, and secondly from 5 sensitive individuals, descending from parental varieties, these fragments being previously subjected to a digestion step, then a ligation step to fix complementary primer adapters having at their end one or more specific nucleotides, one the primers of the 10 pair being labelled for development purposes,
 - separation of the amplification products, by gel electrophoresis under denaturing conditions, and
 - comparison of the electrophoresis profiles obtained with mixtures of fragments derived from 15 resistant descendants and mixtures derived from sensitive descendants, with fragments derived from parental varieties, for the purpose of identifying bands whose polymorphism is genetically linked to the resistance locus, this identification optionally being followed, for validation purposes, by verification on 20 each individual and calculation of the genetic

recombination rate between the marker and the resistance locus.

2. Method according to claim 1, characterized in that the DNA fragments are obtained by digestion of the 5 genomic DNA of resistant plants and of sensitive plants, and their parents, using restriction enzymes.

3. Method according to claim 2, characterized in that as restriction enzymes EcoRI and MseI are used.

4. Method according to claim 2 or 3, characterized 10 in that the restriction fragments are subjected to ligation to fix adapters.

5. Method according to claim 4, characterized in that the fragments obtained are amplified using primer pairs complementary to the adapters whose sequences are 15 respectively GAC TGC GTA CCA ATT C(SEQ ID N°1) and GAT GAG TCC TGA GTA A(SEQ ID N°2).

6. Method according to claim 4 or 5, characterized in that the fragments obtained are amplified using primer pairs having at their end the respective motifs 20 AAC and CAG, ACC and CAG or further AGC and CAG.

7. Method according to any of claims 1 to 6, characterized by the identification of resistance marker bands, M1 and M2, whose size is respectively 510 bp and 140 bp, such as determined by gel 25 electrophoresis under denaturing conditions.

8. Method according to claim 7, characterized in that said marker bands determine a segment of less than 10-15 cM carrying the resistance locus.

9. Method according to claim 8, characterized in 30 that said marker bands are located either side of the locus at less than 5-10 cM.

10. Method according to any of claims 1 to 9, characterized in that it also comprises an isolation step to isolate the identified marker bands.
11. Method according to claim 10, characterized by 5 purification of the isolated marker bands in order to obtain DNA fragments.
12. Method according to claim 11, characterized by cloning of the marker bands into a vector and insertion of the vector in a host cell.
- 10 13. Method according to either of claims 11 or 12, characterized by the recovery and sequencing of the purified, cloned DNA fragments.
14. Method for obtaining markers having high specificity for the locus of a major RYMV resistance 15 gene, characterized in that PCR primer pairs are defined complementary to the sequence of the cloned fragment, specific amplification of this fragment is carried out using these primer pairs, then the amplification products are subjected to migration on 20 electrophoresis gel with or without previous digestion by a restriction enzyme to identify a polymorphism.
15. Polymorphous AFLP bands such as identified by the method according to any of claims 1 to 14 using rice plant DNA.
- 25 16. AFLP bands according to claim 15, characterized in that they are specifically evidenced in a RYMV-sensitive variety, and in the fraction of sensitive plants derived from crossing of this variety with a resistant variety.
- 30 17. DNA sequences corresponding to polymorphous bands according to claim 15 or 16, which can be used to define a segment of chromosome 4 of 10-15 cM carrying the RYMV resistance locus.

18. DNA sequences according to claim 17, characterized in that they correspond to EcoRI-MseI fragments.

19. DNA sequences according to claim 18, 5 characterized by a respective size of 510 bp and 140 bp determined by gel electrophoresis under denaturing conditions.

20. DNA sequences according to any of claims 17 to 19, characterized in that they correspond to sequences 10 flanking the resistance locus and located either side of the latter at 5-10 cM or even at less than 5 cM.

21. DNA sequence, characterized in that it meets SEQ ID N°3.

22. DNA sequence, characterized in that it meets 15 SEQ ID N°9.

23. Cloning vectors, characterized in that they contain sequence SEQ ID N°3 according to claim 21 or sequence SEQ ID N°9 according to claim 22.

24. Host cells, characterized in that they are 20 transformed by vectors according to claim 22.

25. Use of polymorphous bands according to claim 15 or 16 or of DNA sequences according to any of claims 17 to 22 for the identification of resistant phenotypes and transfer of the resistance gene.

26. Fragments of no more than 4-5cM of chromosome 25 4 and polymorphous AFLP bands according to claim 15 or 16 defining a segment of 4-5cM or less carrying the RYMV resistance locus.

27. Use of SEQ ID N°3 or SEQ ID N°9 or of 30 microsatellite markers such as RM252 and RM273, or any other marker such as SEQ ID N°13 of contig 89 of Nipponbare BAC library, and showing polymorphism between a sensitive variety and a resistant variety, to

transfer resistance into a sensitive variety by marker-assisted selection.

28. Use of sequences of contig 89 of Nipponbare library for identifying the sequences of the gene responsible for resistance to the rice yellow mottle virus.